Brief Definitive Report

CORRELATION OF STRUCTURE WITH T CELL RESPONSES OF THE THREE MEMBERS OF THE HLA-DRw52 ALLELIC SERIES

By JACK GORSKI,*‡ CLAUDE IRLE,§ ERIC M. MICKELSON,‖ MICHAEL J. SHEEHY,¶ ANNEMARIE TERMIJTELEN,** CATHERINE UCLA,* AND BERNARD MACH*

*From the *Department of Microbiology, University of Geneva Medical School, Geneva, Switzerland; ‡Immunogenetics Research, The Blood Center of Southeastern Wisconsin, Milwaukee, Wisconsin; the §Institute for Immunology and Hematology-Oncology Unit, La Tour Hospital, Geneva, Switzerland; ‖Puget Sound Blood Center and Fred Hutchinson Cancer Research Center, Seattle, Washington; ¶American Red Cross Blood Service and Department of Medicine, University of Wisconsin, Madison, Wisconsin; and the **Department of Immunohematology and Blood Bank, University Hospital, Lieden, The Netherlands*

The fine structure of polymorphic MHC class II molecules determines their interaction with antigenic peptide and their specific recognition by the TCR, and hence, controls the immune response (1). The class II MHC of individuals identified as DR3, DR5, and DRw6 is characterized by two active DRB loci, DRB1 and DRB3 (2). The allelic series at the DRB3 locus consists of three members, DRw52a, DRw52b (3), and a third member, DRw52c, which is described here. DRw52c represents an intermediate sequence between DRw52a and DRw52b and may have arisen by a gene conversion-like event. The recognition of cells bearing these molecules by a number of alloreactive and antigen-specific, DR-restricted T cell clones was analyzed. HLA-DR is favorable for the study of structure-function correlations since its polymorphism is determined exclusively by the β chain. Using a theoretical model of HLA class II structure (4), different T cell recognition specificities were assigned to specific amino acid positions.

Materials and Methods

Cloning and Sequencing. cDNA was prepared from poly(A) RNA from the homozygous typing cell (HTC), WT46(DRw13; Dw19; DRw52), using a modified Gubler-Hoffman procedure (5), inserted into λ gt10 via linkers, and the cDNAs were screened by plaque filter hybridization using a B3-specific oligonucleotide probe. Positives were purified, subcloned in M13 (6), and sequenced by the dideoxy procedure (7).

Oligonucleotide Typing of Stimulator Cells. Oligonucleotide typing of Southern blot DNA

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samples (8) or of RNA dot blots (9) was performed as described. The regions of the DRw52c molecule used for generating probes are underscored in Fig. 1 *A*.

Stimulation of T Cell Clones. Two types of stimulator cells were used. In most cases the stimulator cells were well characterized EBV-transformed B cell lines (B-LCLs). In three cases PBMC from well characterized individuals were used. In all cases the PBMC were from individuals who had also been used to derive B-LCLs. Activation is measured by cell division (incorporation of [^{3}H]thymidine) and the results are given in counts per minute. Non-DRw52 cells gave background stimulation. The generation and characterization of the clones are described in detail in publications from the respective laboratories (10–13). The reaction of all the clones could be blocked by anti-DR mAbs, including mAb 7.3.19.1 and NDS-10, which are specific for DRB3 locus-associated epitopes, and not by anti-DQ or anti-DP mAbs (10–13).

Results and Discussion

The polymorphism of the HLA-DRB3 locus represents a new allelic series that we have analyzed functionally. Two alleles of DRB3 (52a and 52b) have been described (3, 14), and the sequence of a third allele (52c) is shown in Fig. 1 *A*. This sequence can be derived by alternating between the 52a and 52b allelic sequences (Fig. 1 *B*), suggesting that it may have arisen by a gene conversion–like mechanism, as in the case of other MHC class II genes (3 and references cited therein). The pairwise sharing of polymorphic residues between these three alleles will be useful in functional analysis. These three alleles can be identified by DNA hybridization with locus- and allele-specific oligonucleotide probes (9). The new allele, DRw52c, is in linkage disequilibrium with a subtype of DRw6, called DRw6c or Dw19 (15).

The reactivity of a number of T cell clones responding to HLA DRB3 alleles was compared. The functional reactivities of these T cell clones correlated remarkably

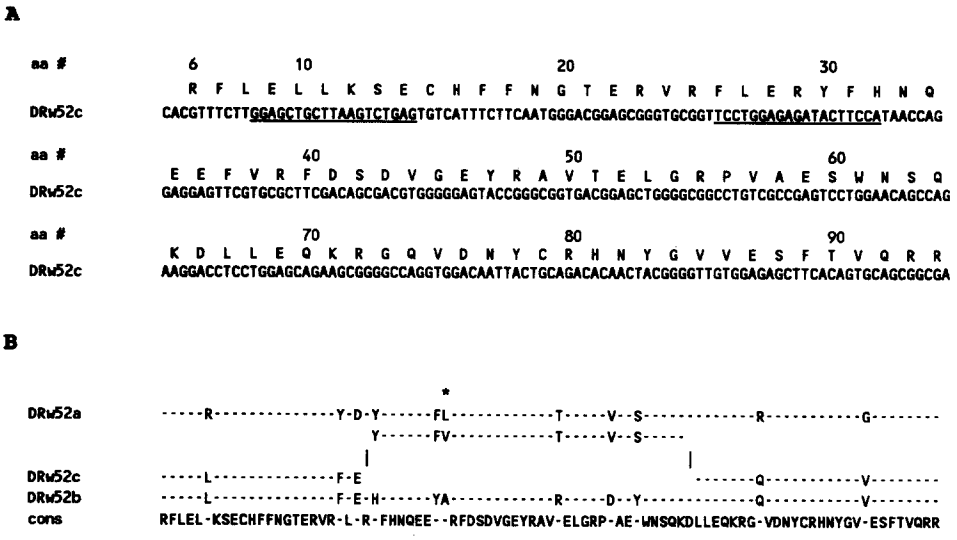

FIGURE 1. (*A*) First domain sequence of the product of the B3 locus from cell line WT46 (DRw13/Dw19) called DRw52c. Underscoring shows where oligonucleotide probes were generated. (*B*) DRw52c may have arisen by a double crossover or gene conversion involving the DRw52a and DRw52b alleles. (*) The only amino acid unique to DRw52c.

TABLE I

T Cell Recognition of DRw52 Alleles

	Representative examples of T cell stimulation						Summary of recognition		
	B-LCL Stimulator specificity			PBMC Stimulator specificity			Stimulator specificity		
Clone ID	DRw52a	DRw52b	DRw52c	DRw52a	DRw52b	DRw52c	DRw52a	DRw52b	DRw52c
C1437	**50,057**	1,620	455	-	-	-	**8/8**	0/10	0/6
C21	-	-	-	165	**24,700**	220	0/4	**5/5**	0/3
B33-25	235	**18,661**	445	-	-	-	0/18	**18/18**	0/8
B33-30	1,429	**17,168**	**16,307**	-	-	-	0/18	**18/18**	**8/8**
C6	**36,244**	361	561	-	-	-	**4/4**	0/16	0/6
E3	573	**14,400**	812	-	-	-	0/7	**16/16**	0/7
ZUK16	519	4,221	**29,306**	-	-	-	0/6	2/16*	**7/7**
16 + TT	-	-	-	**10,439**	1,064	**3,995**	**10/10**	0/14	**2/2**
10 + TT	-	-	-	580	**71,759**	**8,995**	0/10	**14/14**	**2/2**

The clones are alloreactive T cells, except clones 16 and 10, which are restricted to homologous MHC and tetanus toxin. Proliferation is shown as incorporation of [^{3}H]thymidine and positive responses are in bold. On the right, the summary of T cell activation by all oligo-typed DRw52 cells tested is shown as the ratio of reactive/tested cells. The B-LCLs represent a mix of cells local to the laboratories involved and a selection of cells from the Tenth International Histocompatibility Workshop. Restriction of the clones by HLA-DR molecules has been shown previously for clones 10 and 16 (10), clones C6, E3, and and ZUK16 (11), clones B33-25 and B33-30 (12), and clones C1437 and C21 (13) by mAb blocking studies.

* T cell clone ZUK16 always showed a low level response to DRw25b-expressing cells. In two cases this response level was equivalent to DR52c responses.

with the presence of the 52a, b, and c alleles, defined structurally. An example of results obtained in typical experiments with each of the T cell clones (either in alloreactivity or in autologous antigen presentation) is shown in Table I (left side), and the results of all experiments are also summarized in Table I (right side). Three types of T cell clones are thus identified: (*a*) clones that are stimulated by only one DRB3 allele (C1437, C21, B33-25, C6, and E3); (*b*) clones with a predominant response to one allele and a definite crossreactivity with another allele (ZUK16, 10, 16); and (*c*) a clone that responds equally well to two alleles (B33-30). The pattern of T cell responses can now be correlated with the amino acid sequences of the three DRw52 alleles, in order to map residues important for T cell recognition.

A theoretical model has recently been proposed for class II structure based on the homology between individual domains of class I and class II molecules (4). In this model, side chains on the α helix are postulated to either contact the antigenic peptide or the TCR, whereas the sidechains on the β sheets contact antigen alone.

Because the T cell clones can discriminate between the three DRB3 alleles, we indicated in Fig. 2 the amino acids that distinguish all possible pairs among these three alleles. These polymorphic positions appear as clusters on the DR molecule. Depending on which two alleles are being compared, different regions of the DR molecule determine the specificity (Fig. 2, *A* and *B*). Thus, the allorecognition of DRw52c by a DRw52a T cell involves polymorphism in the central portion of a molecule (Fig. 2 *A*), whereas it involves a more exterior portion when recognized by a DRw52b T cell (Fig. 2 *B*). The same is true when a DRw52c T cell recognizes DRw52a or DRw52b molecules. Two such clones are shown in Table I (C6 and E3). Furthermore, DRw52a T cells directed against DRw52b could represent a spectrum

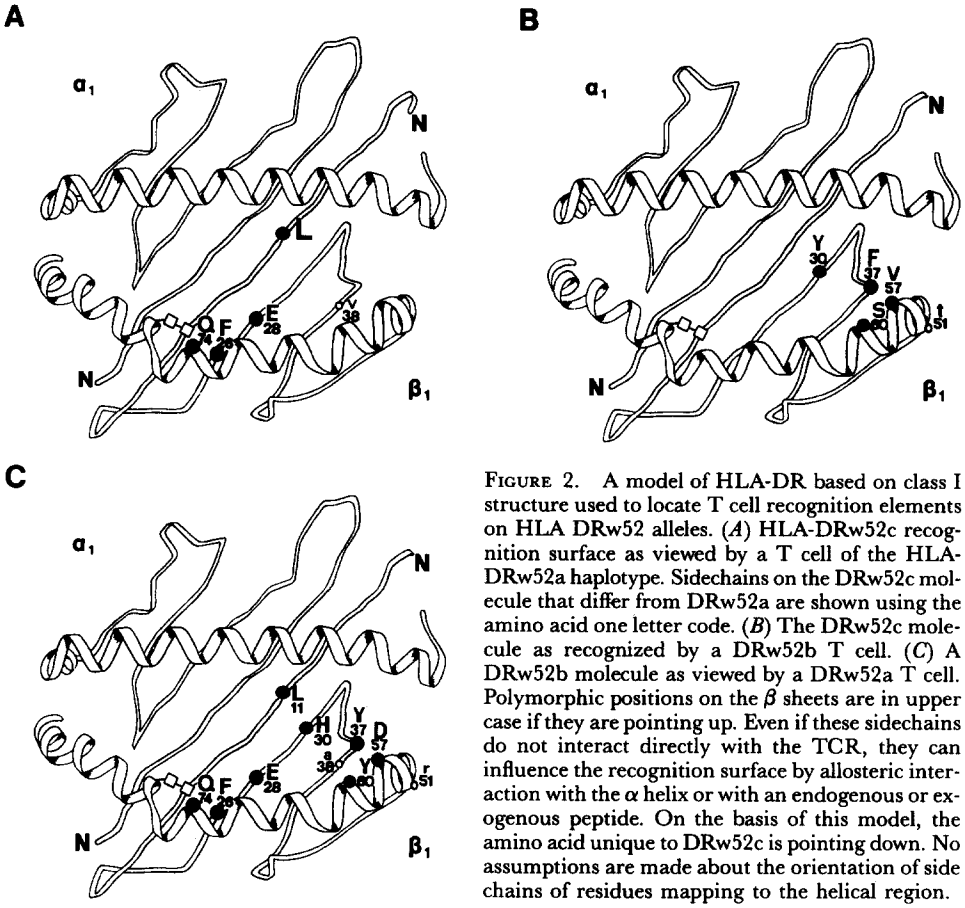

FIGURE 2. A model of HLA-DR based on class I structure used to locate T cell recognition elements on HLA DRw52 alleles. (*A*) HLA-DRw52c recognition surface as viewed by a T cell of the HLA-DRw52a haplotype. Sidechains on the DRw52c molecule that differ from DRw52a are shown using the amino acid one letter code. (*B*) The DRw52c molecule as recognized by a DRw52b T cell. (*C*) A DRw52b molecule as viewed by a DRw52a T cell. Polymorphic positions on the β sheets are in upper case if they are pointing up. Even if these sidechains do not interact directly with the TCR, they can influence the recognition surface by allosteric interaction with the α helix or with an endogenous or exogenous peptide. On the basis of this model, the amino acid unique to DRw52c is pointing down. No assumptions are made about the orientation of side chains of residues mapping to the helical region.

of responses, depending on which area of the DRw52b antigen is involved in determining the recognition (Fig. 2 *C*). Some of these recognition sites would be unique, while others may be shared with DRw52c since the central polymorphic regions of DRw52c and DRw52b are identical. In an example of such a 52a vs. 52b priming, two T cell clones (B33-25 and B33-30) were derived. B33-30 recognizes both 52b and 52c equally and thus this shared region is instrumental in controlling the TCR recognition. This example of shared recognition provides a molecular basis for cases of public T cell alloresponses. The ease of generating public alloreactive clones may in part explain the high levels of alloreactive responses as compared with antigen-specific responses. It follows from these data that the stimulation of different T cells is controlled by different regions, or polymorphic amino acid clusters, of the HLA-DR molecule. Some public T cells appear to be restricted by a polymorphic cluster, even if it is present on more than one MHC allele.

Another feature of DR molecules relevent to public recognition by T cells is the lack of α chain polymorphism. This results in a large portion of the external face of the DR molecule (Fig. 2, upper left portion of models) being identical between

alleles. T cells whose TCRs are predominantly restricted by this region would exhibit public recognition.

The differences observed in this allelic series fall either in the interhelical region of the β sheet or at exposed positions on the α helix itself. In the two simplest cases (Fig. 2, *A* and *B*) the recognition structure involves two amino acids in the interhelical grove and two amino acids on the α helix itself. In the case of alloreactive responses, the specificity-determining polymorphic positions in the β sheets are interesting, since according to the class I model, it is unlikely that they can come into direct physical contact with the TCR. Nevertheless, amino acid differences at these positions could modulate recognition by the TCR by conformational or allosteric effects on the exposed α helical portion of the molecule, or more likely, by controlling the binding of an exogenous or endogenous peptide to the groove of the class II molecule. Polymorphic residues in the helical portion of the molecule could contact TCR directly or could again act indirectly through peptide binding. Recent results implicate the involvement of antigenic peptides even in alloreactive responses (16–18 and Gorski, J., and D. Eckels, manuscript in preparation) as well as in antigen presentation.

The DRB3 allelic series is of special interest since at every polymorphic position, two of the alleles are identical. This has enabled us to focus on small structural changes affecting T cell recognition. The alleles studied here are the natural products of evolution and therefore complement in vitro mutagenesis studies.

Finally, the stimulation of both alloreactive and antigen-restricted T cells by alleles of the B3 locus in the DRw52 family implies that these alleles are important in the immune response. Indeed a major role of the HLA-DR B3 products in T cell responses to clinically important allergens has recently been documented (Lamb, J., et al., manuscript submitted for publication). Thus, this allelic series, whose existence was only formally demonstrated recently (2) and which is unresolvable by current serological techniques (8), must be considered when evaluating the role of HLA class II molecules in transplantation, response of infectious agents, or autoimmunity.

Summary

A third allele at the DRB3 locus, DRw52c, represents an intermediate sequence between DRw52a and DRw52b and may have arisen by a gene conversion-like event. The recognition of cells bearing these molecules by a number of alloreactive and antigen-specific DR-restricted T cell clones was analyzed. On the basis of a theoretical model of HLA class II structure, distinct amino acid clusters have been identified as motifs controlling TCR recognition. These are located both in the cleft and in the α-helical edge of the MHC class II recognition platform. Motifs shared between two alleles may restrict public T cell clones.

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